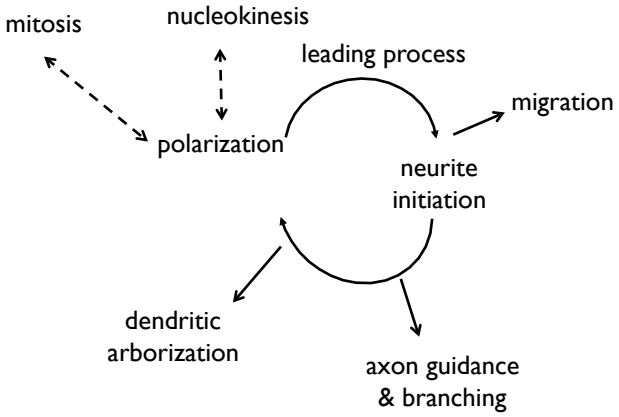
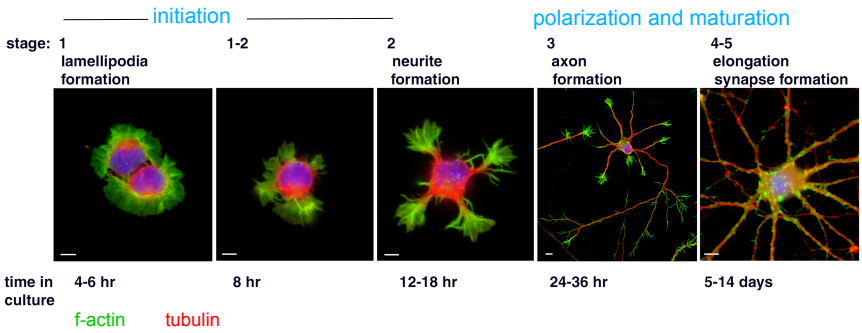
# Neuronal polarity, axon elongation and axon branching

**Milestones in neuronal development**



## Neuronal maturation

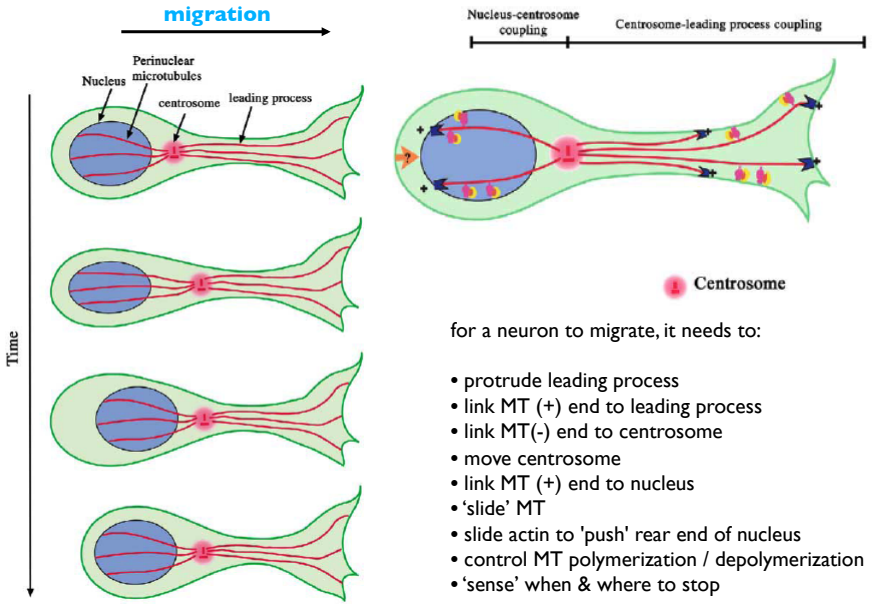



- stage 1: "spherical" neuron
- stage 2: neurons extend several neurites
- stage 3: one neurite accelerates its growth rate and matures to form the axon.
- stage 4: dendrites begin to elongate and branch
- stage 5: synaptogenesis

## Leading process formation and axon elongation

Some radially migrating neurons
leave an axon at the
ventricular surface
as they migrate.

## Cytoskeletal dynamics during neuronal migration

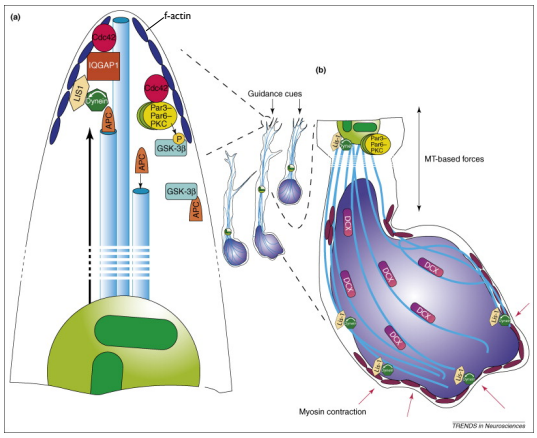



for a neuron to migrate, it needs to:

- protrude leading process
- link MT (+) end to leading process
- link MT(-) end to centrosome
- move centrosome
- link MT (+) end to nucleus
- 'slide' MT
- slide actin to 'push' rear end of nucleus
- control MT polymerization / depolymerization
- 'sense' when & where to stop

Tsai & Gleeson (2005) Neuron

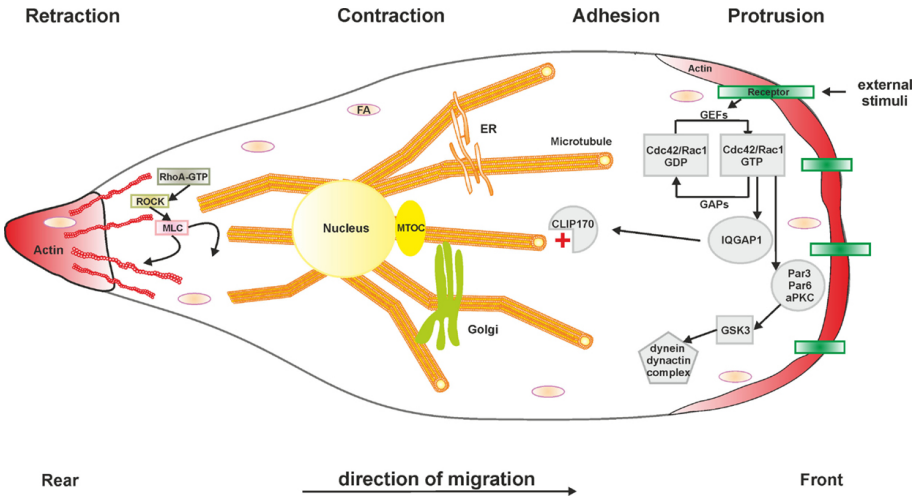
## Molecules involved in neuronal migration




- Dynein: (-) end directed motor protein
- APC: MT (+) end binding & stabilizing, also bind and is regulated by GSKβ
- GSKβ: regulatory kinase
- LIS1: Lissencephaly gene, "regulator/adapter", interacts with multiple proteins including dynein, IQGAP & cdk5
- DCK: Doublecortin, MT bundling protein
- IQGAP: cdc42 GAP (GTPAse Activating Protein)
- PAR3 & PAR6: polarity genes that bind to and localize PKC

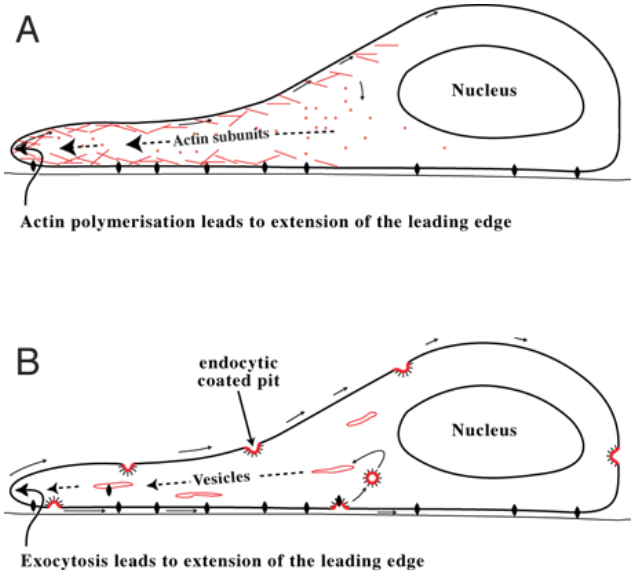
Higginbotham, H.R., and Gleeson, J.G. (2007).

## Molecules involved in neuronal migration are similar to those involved in neurite elongation

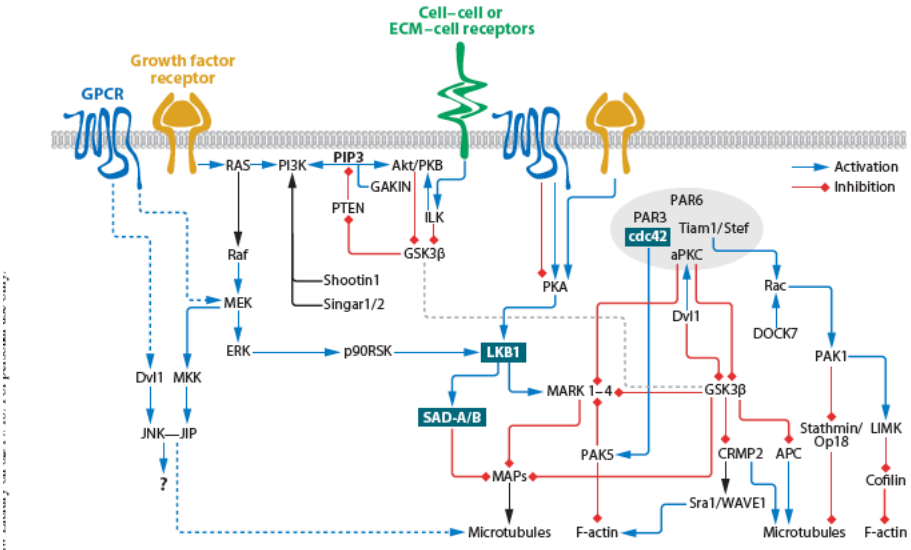



Front. Immunol., 14 September 2015

## Two competing (?) views of cell migration

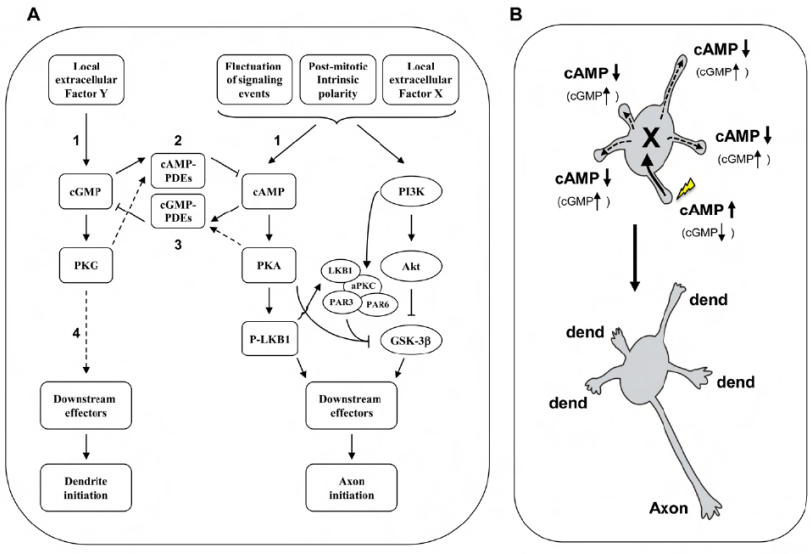


Signaling Pathways that determine neuronal polarity ultimately lead to changes in the actin and microtubule cytoskeleton



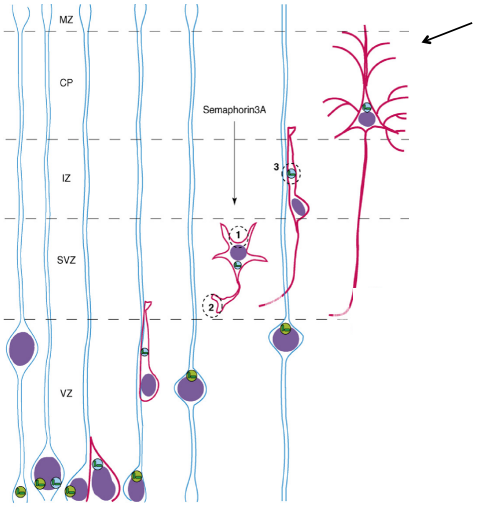

Barnes & Polleux (2009) Annu rev Neurosci

PKA and PKG play important, reciprocal roles in axon/dendrite formation, even in dissociated pyramidal neurons




Shelly et al. (2010) Science

A combination of extrinsic cues and the intrinsic polarization of the neuron interact as immature neurons are undergoing migration to determine the location and orientation of the axon and apical dendrite.



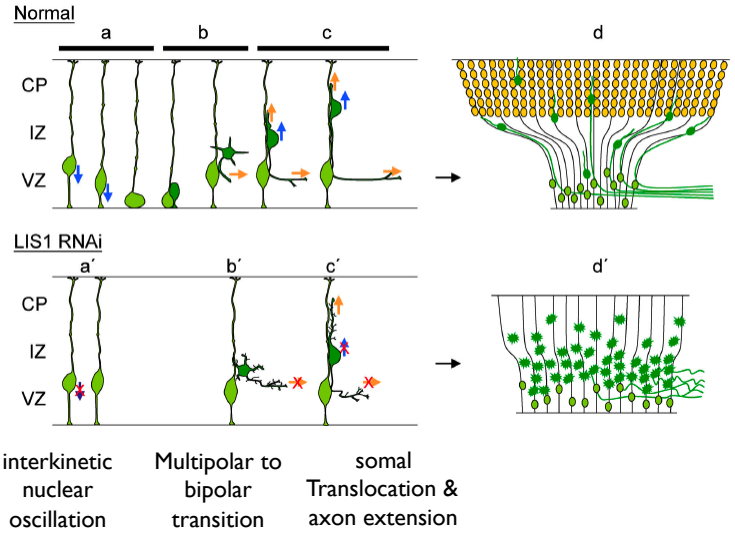

Dendrites:

high levels of soluble guanyl cyclase (SGC, makes cGMP) = attraction to Sema3a

Axon:
low SGC, repulsed by Sema3A

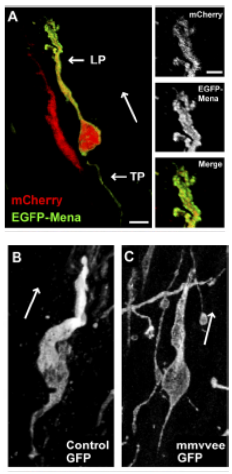
Polleux et al (2000) Nature
Higginbotham and Gleeson (2007)

## Lis1 is involved in multiple stages of neuronal migration and axon elongation

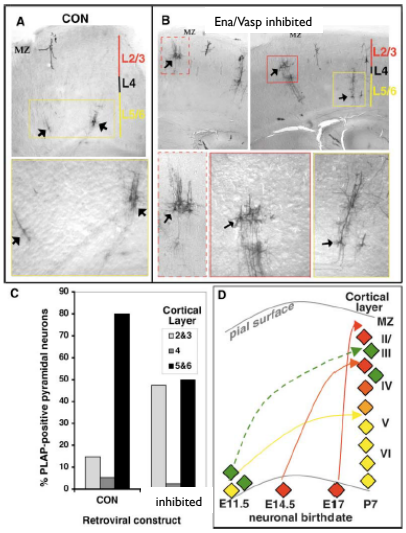



Tsai et al. (2005) JCB 170(7):935-945

**Ena/VASP proteins are required for neurite initiation, but not leading process formation or neuronal migration per se. Nonetheless, they regulate neuronal positioning.**




Kwiatkowski et al. (2007. Neuron, 56(3), 441–455

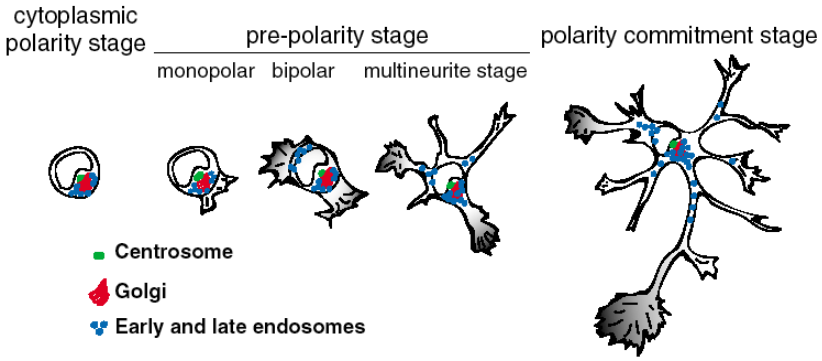



Goh et al. (2002).. Current Biology .12(7), 565–569.

Neuronal polarization:

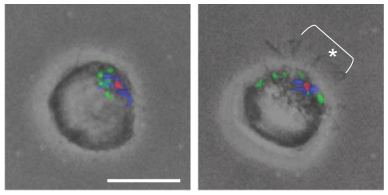
What determines which neurite becomes the axon?

Some studies have shown that the axon is most likely to be formed by the first-formed neurite, followed next most frequently by the neurite at the opposite side, which is the second-formed neurite. This bipolarity may be due to the intrinsic orientation of the centrosome and Golgi apparatus, and their influence on transport of materials into neurites.

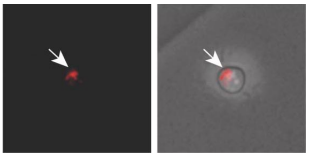



Several extrinsic manipulations of cultured hippocampal neurons can determine axonal identity. These manipulations include pulling on a stage 2 neurite, patterning the substrate with natural molecules or releasing molecules in a gradient.

## Does centrosome localization determine neuronal polarity?

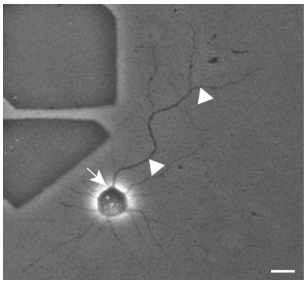



centrosome, Golgi, endosome

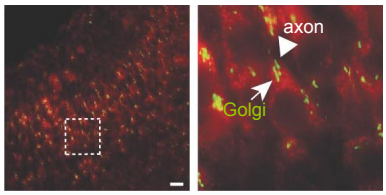


centrosome before neurite initiation

'protrusions' (*) initiate adjacent to the centrosome'



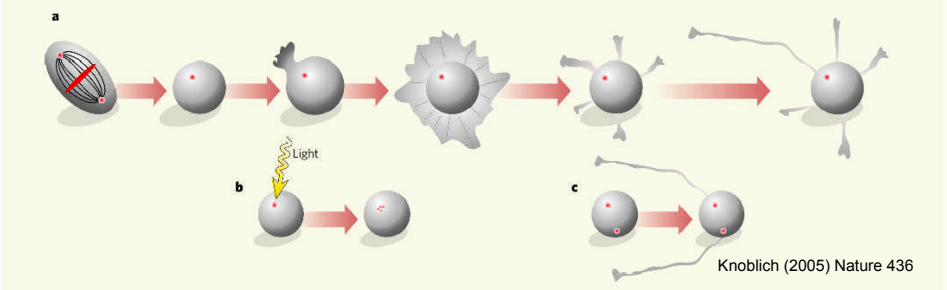
after axon formation




E18 rat hippocampus, coronal section

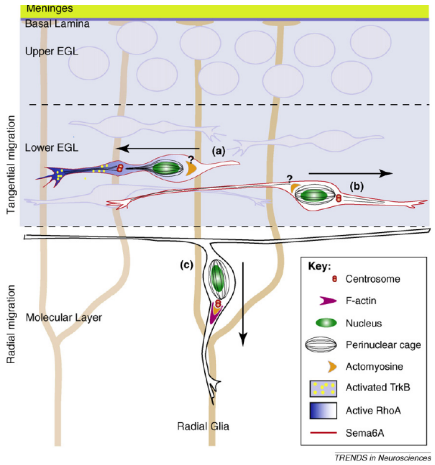
de Anda et al (2005) Nature

## Data indicate that centrosome position may determine where the axon forms. But...



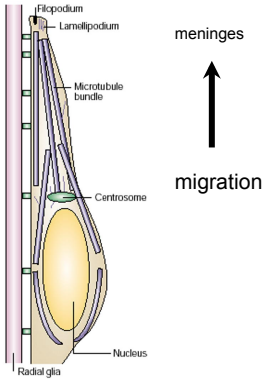

Knoblich (2005) Nature 436

## Centrosome position may actually determine which process is currently elongating the most

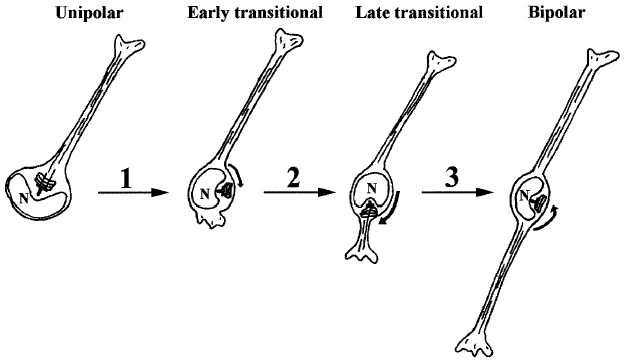



During radial migration of granule cells in the cerebellum, the leading process becomes the dendrite

In the cortex, the centrosome is positioned on the pial side of the nucleus during radial migration, while the axon forms on the ventricular side of the nucleus.



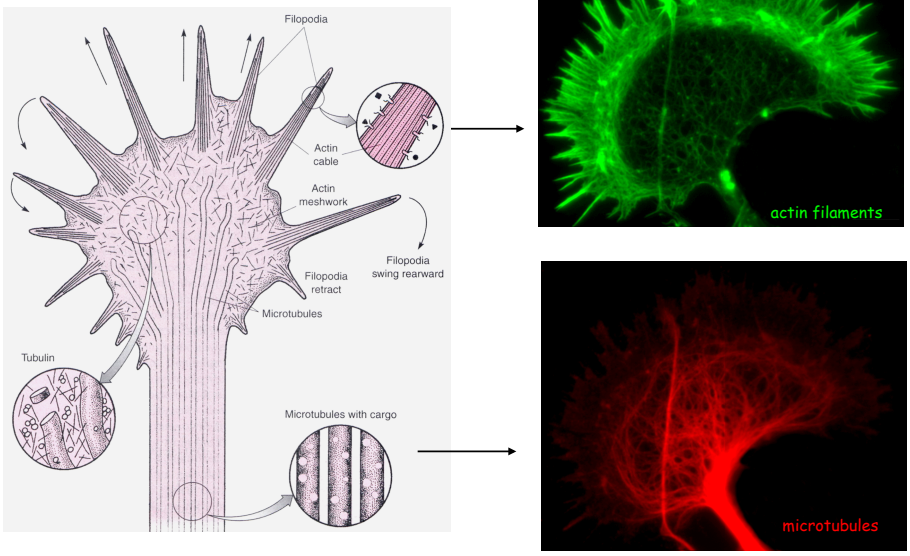
## Re-orientation of the centrosome/Golgi in cerebellar granule cells



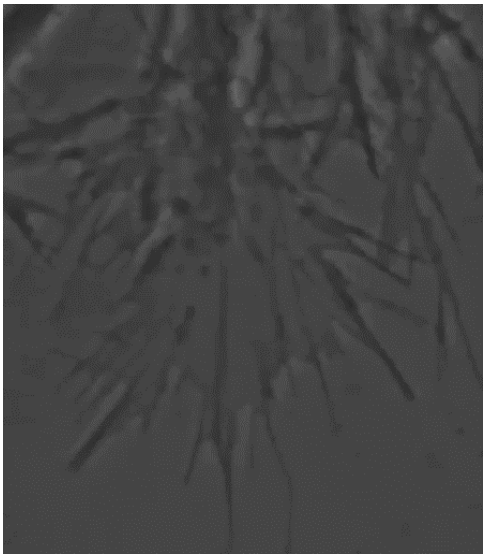

Zmuda and Rivas (1998) Cell Motility and the Cytoskeleton

What drives growth cone motility and axon elongation?

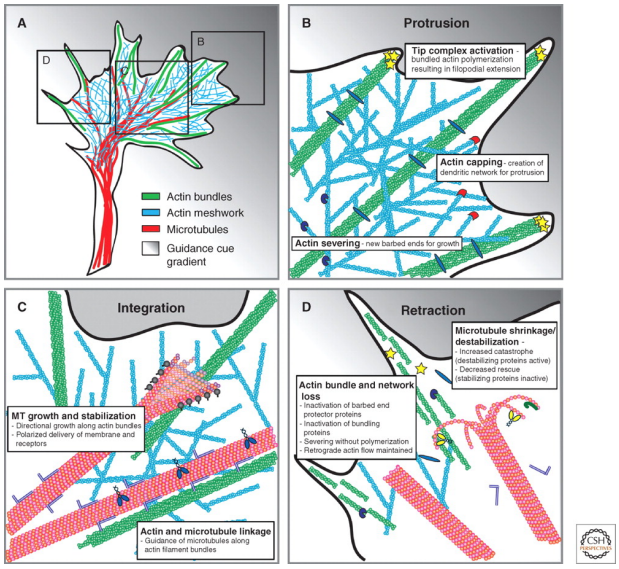
## The growth cone




**The leading margin of the growth cone undergoes continuous protrusion and withdrawal of filopodia and veils. This involves dynamic reorganization of the actin filament and microtubule networks.**

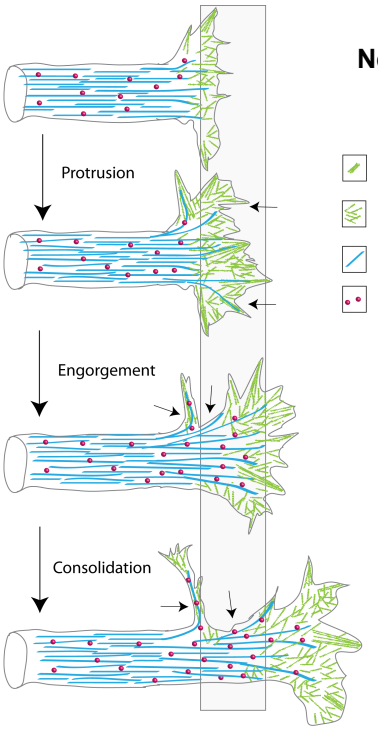


## Coordinating actin and microtubule dynamics in neurite initiation and elongation






Dent E W et al. Cold Spring Harb Perspect Biol 2011;3:a001800




### Neurite elongation is a 3 step process

Bundled F-actin

F-actin meshwork

Microtubule

Vesicles

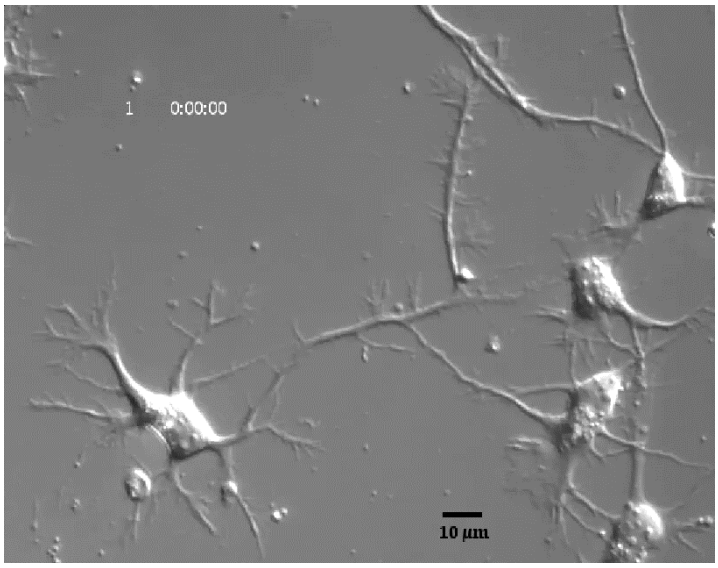
**PROTRUSION**
Actin polymerization drives membrane expansion forward

**ENGORGEMENT**
Microtubules advance via transport and polymerization. Organelles move forward.

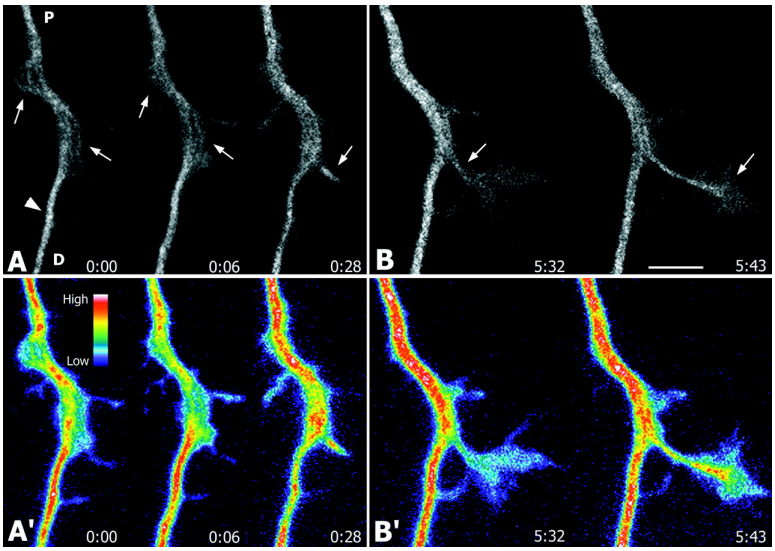
**CONSOLIDATION**
Cortical tension draws the neurite shaft forward.

- Inhibit MT polymerization or transport, axonal elongation stops/retracts.
- Inhibit actin polymerization or myosin tension, axonal elongation continues but is slower and disordered.

## Axon elongation & branch formation

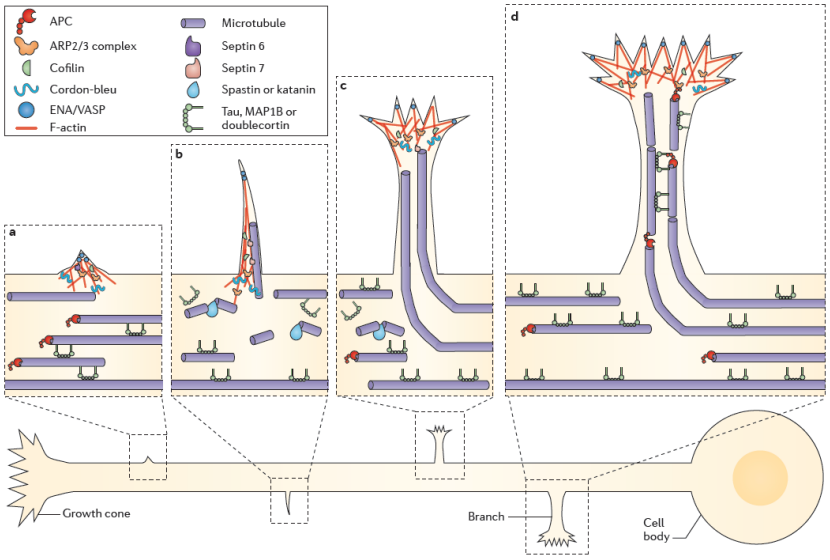



## Microtubule density before and during axonal branch formation



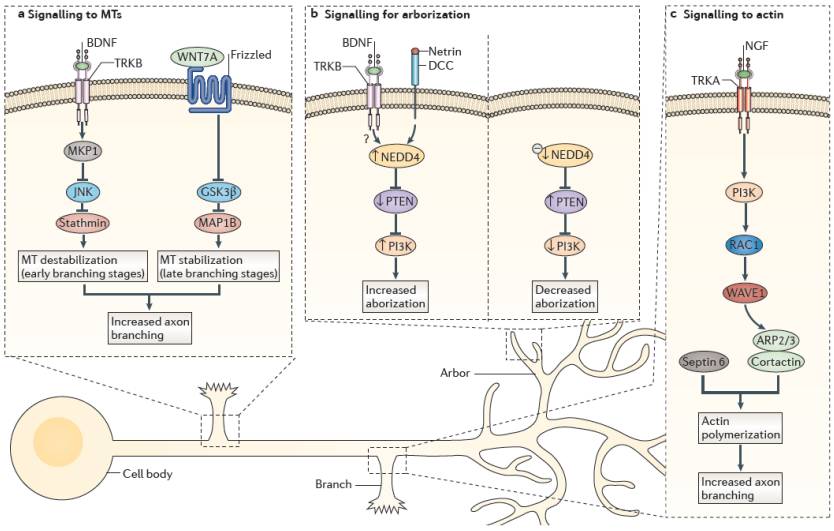

Dent and Kalil J Neurosci. 2001 Dec 15;21(24):9757-69

## Cytoskeletal dynamics during axon branching




Kalil and Dent 2014 Nat Rev Neurosci

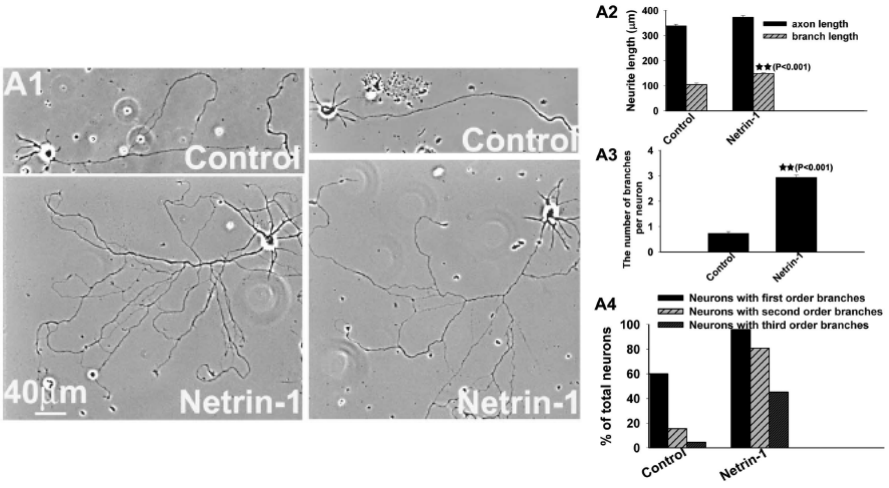
## Signalling during axon branching




Kalil and Dent 2014 Nat Rev Neurosci

## Netrin-1 induces axonal branching

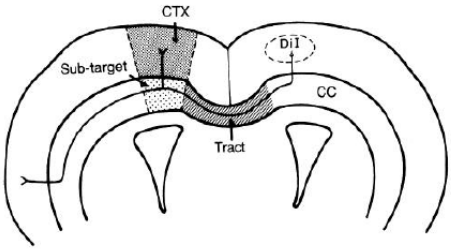
**...what types of cytoskeleton regulation might be involved?**

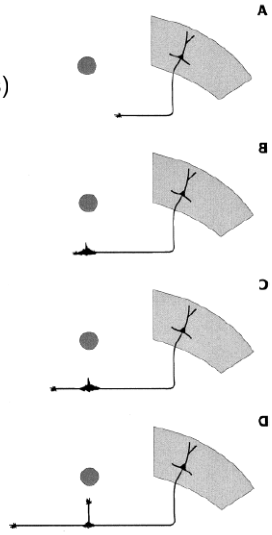



Tang & Kalil (2005) J. Neuroscience

## Correlation between pausing and branching

in vivo growth cones appear to:
- pause: retrograde flow = anterograde protrusive
- enlarge (increased MT based transport by (+) end motors)
- exhibit dynamic protrusion/retraction
- leave 'something' behind
- branch formation occurs sometime later




Halloran or Kalil (1994)

## Neuronal polarity, axon elongation and axon branching

- Determination of neuronal polarity involves the mitosis, neuronal migration and neurite initiation
- These processes cannot necessarily be divided into discrete stages
- Axon branching recapitulates many aspects of neurite initiation and may be triggered by by both trophic and guidance factors
- Both cell intrinsic and extrinsic factors are involved